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Environmental Effects of Dredging Technical Notes

INFLUENCE OF ENVIRONMENTAL VARIABLES ON BIOACCUMULATION OF MERCURY

PURPOSE: This note examines the effects of environmental factors on the bioavailability of mercury from sediment and describes results of a laboratory experiment to assess the influence of temperature, salinity, and suspended sediment on bioaccumulation of mercury in estuarine clams and killifish.

BACKGROUND: Public laws regulating dredged material disposal (Section 404 of the Clean Water Act and Section 103 of the Ocean Dumping Act) require ecological evaluation prior to the permitting of operations. Assessment of the potential for bioaccumulation of chemical contaminants in sediment, including heavy metals, is required as part of the evaluation process. Metals can represent significant sediment contamination in the vicinity of industrial and commercial point sources. Mercury, in particular, enters the aquatic environment in various forms from chloralkali and instrumentation plants, paints, pulp and paper manufacture, agricultural sources, and other nonpoint sources such as atmospheric deposition (Khalid et al. 1977). Because sediment serves as a sink for mercury, dredging and disposal operations can affect the bioavailability of mercury to aquatic organisms. In general, metals in sediment have low bioavailability in reduced environments such as aquatic disposal sites, but may be highly bioavailable in upland disposal sites where the dredged material is subject to drying and oxidation.

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Introduction

Mercury is among the most toxic of the heavy metals and thus can greatly concern regulators faced with the dredging and disposal of mercury-contaminated sediment. Acute toxicity tests have demonstrated the lethality of mercury to various aquatic organisms, including polychaetes (Warren 1976), adult and larval crabs (Vernberg and Vernberg 1972; McKenney and Costlow 1981, 1982), and daphnids (Khangarot, Ray, and Chandra 1987), especially under conditions of environmental stress. Mercury can exist in various forms in the

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environment, including inorganic mercury (Hg^0, Hg^{1+}, Hg^{2+}) and various organic complexes such as the highly toxic methyl mercury. Besides lethality, various sublethal effects in aquatic organisms have been attributed to methyl mercury exposure, notably interference with development or regeneration in tadpoles, fish, and crabs (Chang, Reuhl, and Dudley 1974; Weis and Weis 1978; Callahan and Weis 1983).

Methylation of mercury can occur either through biotic or abiotic processes (Nagase et al. 1982, 1984), although the environmental significance of abiotic methylation is probably minor (Berman and Bartha 1986b). In the aquatic environment, methylation of mercury is likely to occur wherever there is microbial activity, e.g., in the sediment, water column, and digestive tract of fish (Rudd et al. 1983). Methylation of mercury can occur in either anaerobic sediment (Hammer, Merkowsky, and Huang 1988) or aerobic sediment (Fagerstrom and Jernelov 1970). High sulfide concentrations inhibit methyl mercury production (Berman and Bartha 1986a).

Various investigators have reported high mercury concentrations in sediment and organisms in the vicinity of mercury pollution sources (Kiørboe, Møhlenberg, and Riisgård 1983; Mikac et al. 1985). Mercury concentrations in fish and crustaceans taken from the New York Bight, nearby New Jersey, and Long Island Sound ranged from 0.08 to 2.3 parts per million (ppm) (Roberts, Hill, and Tifft 1982). However, animals exposed to New York Harbor sediment in laboratory studies did not accumulate mercury even though sediment mercury concentrations ranged from 2 to 35 ppm (Rubinstein, Lores, and Gregory 1983). These investigators proposed that high organic or sulfide content in the sediment bound the mercury and rendered it unavailable. Weis, Weis, and Bogden (1986) reported no correlation between mercury bioaccumulation in the killifish *Fundulus* and mercury concentrations in the sediment to which the fish were exposed.

Bioaccumulation of mercury from sediment by aquatic organisms can be influenced by a number of environmental factors, including temperature, salinity, dissolved oxygen, pH and alkalinity, suspended sediment, organic carbon content of the sediment, and presence of other elements. Of these factors, the last may be one of the most important. Sulfide (S^{2-}), in particular, may be the primary regulator of Hg^{2+} activity in natural waters (Bjornberg, Håkanson, and Lundbergh 1988). If the pH is high or the redox potential is low, then sulfide activity will be high and virtually all mercury will be

precipitated as very poorly soluble HgS. Conversely, at lower pH or higher redox potential, sulfide activity will be lower, and mercury activity and bioavailability will be higher. Two other elements, selenium and tellurium, interact with mercury in the same way as sulfide.

The influence of temperature and salinity on mercury bioavailability and toxicity is not well understood, and diverse observations have been reported. Parks, Sutton, and Hollinger (1984) noted that increases in water temperature result in increases in total mercury and methyl mercury concentrations in water. Weis, Weis, and Bogden (1986) reported a fivefold increase in mercury uptake by *Fundulus* during the summer months in a mercury-contaminated tidal creek, whereas Cossa and Rondeau (1985) found mercury bioaccumulation in mussels to be lower in summer than during other seasons. Smith, Green, and Lutz (1975) found temperature to have no effect on the rate of mercury uptake or elimination by freshwater clams. Olson and Harrel (1973) reported higher toxicity of mercury to the estuarine clam *Rangia cuneata* in fresh water than in salinities of 5.5 and 22 parts per thousand (ppt). In a factorial experiment, Khayrallah (1985) observed that the toxicity of mercury to amphipods was directly related to concentration and temperature, but inversely related to salinity and age of the test animals.

Several investigators noted increased mercury accumulation in biota at low dissolved oxygen levels (Weis, Weis, and Bogden 1986; Hammer, Merkowsky, and Huang 1988). Björnberg, Håkanson, and Lundbergh (1988) postulated that this phenomenon may be due to increased methylation of mercury under anoxic conditions.

Mercury partitions readily from water to suspended sediment (Sayler and Colwell 1976), and also to the organic fraction in oxidized surface layers of sediment (Langston 1982). In either case the mercury may be largely unavailable to organisms (Langston 1986, Rudd and Turner 1983). Rudd et al. (1983) noted that mercury methylation and bioaccumulation are inversely related to the concentration of mercury-binding particulates present. Breteler, Valiela, and Teal (1981) found the highest concentrations of mercury in animals living in marsh sediments lowest in organic matter. Contradictory data suggest that humic substances transfer mercury from sediment to the water phase and then to biota (Surma-Aho et al. 1986); thus, high humic content in sediment may be linked to high mercury content in biota (Björnberg, Håkanson, and Lundbergh

1988). Mercury in humic particles can be converted to bioavailable forms by microbial methylation.

The laboratory experiment described in this note was designed to assess the influence of temperature, salinity, and mercury-contaminated suspended sediment on bioaccumulation of mercury by clams (*Rangia cuneata*) and killifish (*Fundulus heteroclitus*). Other environmental factors such as dissolved oxygen and pH were held constant or nearly constant.

Materials and Methods

The experimental system used was the Flow-through Aquatic Toxicology Exposure System (FATES) developed at the US Army Engineer Waterways Experiment Station (WES). This system consists of 24 flow-through cylindrical aquaria having round bottoms and a 75-ℓ capacity. The entire system is controlled by a microcomputer that interfaces with valves and other mechanical equipment via microprocessor-based data acquisition and control hardware. Temperature, salinity, suspended sediment loads, and water flow-through rates are all controlled and may be set to whatever parameters are needed in the experiment. The system also incorporates a light level timer for day/night simulation. Commercially available artificial sea salt is mixed with aged, dechlorinated tap water when saltwater experiments are conducted in FATES.

Test sediment was collected from a mercury-contaminated tidal creek in the northeastern United States and held at 4° C until used. The sediment was diluted with deoxygenated water and mixed with a high-speed, shear-type mixer to provide a uniform, small particle-size slurry. The slurry was pumped into a stainless steel, cone-bottom hopper and kept in constant circulation to prevent settling. Once the hopper was pressurized with approximately 2 psi of argon gas to retard oxidation, the slurry was then ready for use in FATES.

The level of suspended sediment in each aquarium was maintained by a computer-controlled feedback system. A transmissometer head, located in each aquarium, measured suspended sediment level by light transmission every 10 to 15 min and if the level was low, an injector valve was activated to pulse a small amount of slurry into the aquarium. A recirculating pump dispersed the slurry uniformly throughout the aquarium. Average suspended sediment concentrations were maintained near the targeted levels.

The water flow-through volume in each aquarium was computer controlled

at 300 mℓ/min, allowing 95 percent water replacement every 12 hr to maintain high water quality. Temperature of the aquaria was maintained with a heat-exchanger system. The computer checked the temperature of the heat exchangers several times every minute and added hot or cold water as needed to the heat exchangers to keep the temperature constant. All 24 aquaria were sequentially sampled every 6 hr for temperature, dissolved oxygen, pH, and conductivity. These data were written to a computer disk file and printout for later analysis.

The experiment consisted of six 7-day (168-hr) exposures in various temperature and salinity combinations (Table 1). During each exposure, suspended sediment concentrations were maintained in individual aquaria at nominal levels of 0, 5, 15, 25, 50 and 100 mg/ℓ. Each aquarium contained 1 to 2 ℓ of bedded sediment below a screen to prevent test animals from having direct contact with the sediment. In addition three control aquaria contained clean pea gravel with no bedded or suspended sediment. The assignment of controls and suspended sediment levels to aquaria was done in a random manner.

Table 1

Environmental Conditions Used for Each of 6 Runs

Run No.	Salinity ppt	Temperature °C
1	6.0	12
2	6.0	25
3	2.0	25
4	0.5	25
5	2.0	12
6	0.5	12

Killifish and clams were acclimated to experimental conditions for at least 10 days before each exposure. Tissue samples were taken when the animals were received at WES to determine any background residues of contaminants. Before the beginning of each run, environmental parameters were checked to verify that they were within the ranges needed. Once these were found to be acceptable, approximately 25 fish and 30 clams were placed in each of the 24 aquaria and Day 0 tissue, culture water and slurry samples were

taken. During the next 7 days, the animals were not fed, but were checked daily and any dead ones removed. Total suspended solids were determined gravimetrically to verify the levels in each aquarium and unfiltered water samples were taken from each aquarium during the exposure. On Day 7 the animals were removed from each aquarium and allowed to depurate for 24 hours in clean flowing water at the same salinity as the run. The clams were then shucked and tissues of both clams and fish were frozen in separate glass jars by aquarium.

At the end of each exposure, bedded sediment was removed and stirred, and the aquaria were cleaned and refilled with water. The bedded sediment was placed back into each aquarium in preparation for the next run.

Water and tissue samples were analyzed for mercury using the cold vapor atomic absorption technique (American Public Health Association 1985) except for sample preparation. Water samples were prepared by continuous stirring while two 100-mℓ aliquots were removed. The first subsample was filtered through a 0.45-μm membrane filter while the second subsample was unfiltered. The subsamples were transferred to biological oxygen demand (BOD) bottles and analyzed by cold vapor atomic absorption for total mercury content. Tissue samples were prepared after thawing and homogenizing. Weighed subsamples were placed into digestion tubes, treated with nitric acid, and heated to 125° C until all tissue was dissolved (Evans, Johnson, and Leah 1986). The resulting solutions were evaporated to approximately 1.5 mℓ and diluted with distilled water to a known volume. Each subsample was transferred to a BOD bottle and analyzed by cold vapor atomic absorption. Appropriate US Environmental Protection Agency (USEPA) water and tissue quality control samples were run to verify proper functioning of equipment and procedures.

Data were analyzed using the SAS General Linear Models (GLM), Regression (REG), and MEANS procedures (SAS 1985). Values below detection limits were set equal to the detection limits for inclusion in analyses. Prior to analysis of variance (anova) or analysis of covariance (ancova), the assumption of homogeneity of variances was tested using Levene's test (Brown and Forsythe 1974), and a data transformation or nonparametric procedure employed if needed. Analyses of covariance also included a test of the ancova assumption of parallelism. Following significant anovas, means were compared using Duncan's multiple range test (two means), the Waller-Duncan k-ratio t-test (three or more means), or orthogonal contrasts (preplanned comparisons).

Functional regression equations were determined using geometric mean regression analysis when the independent variable could not be specified without error (Halfon 1985, Ricker 1984).

Results

Temperature and salinity measured in the aquaria during the six runs (Table 2) were close to the predefined experimental conditions listed in Table 1. Dissolved oxygen (DO) and pH were not controlled during the experiment but remained stable throughout all runs. DO was 9 to 10 mg/ℓ and pH was approximately 8 in all runs (Table 2).

Table 2

Mean Measured Physical Parameters for Each of the Six Runs

Physical Parameter	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Temperature, °C	11.22 (0.80)	25.60 (0.80)	24.82 (0.46)	24.44 (0.37)	10.95 (0.60)	12.13 (1.06)
Salinity, ppt	6.0 (0.26)	6.6 (0.11)	2.4 (0.27)	1.0 (0.03)	2.1 (0.30)	1.0 (0.00)
Dissolved oxygen, mg/ℓ	9.37 (0.13)	9.50 (0.13)	9.60 (0.48)	9.62 (0.27)	9.56 (0.45)	10.03 (0.55)
pH	8.27 (0.06)	8.33 (0.13)	7.97 (0.07)	7.84 (0.06)	7.93 (0.05)	7.87 (0.10)
Total Suspended Sediment (TSS), 5 mg/ℓ	13.3 (6.1)	13.4 (9.9)	4.7 (2.5)	8.2 (3.2)	13.2 (7.5)	14.7 (6.0)
TSS, 10 mg/ℓ	18.1 (7.5)	14.8 (7.3)	8.3 (1.9)	17.8 (8.8)	18.1 (5.9)	18.8 (8.6)
TSS, 15 mg/ℓ	32.5 (9.0)	27.3 (8.6)	20.4 (4.8)	19.8 (5.3)	29.0 (9.8)	29.3 (6.4)
TSS, 25 mg/ℓ	30.7 (12.1)	35.8 (15.1)	25.7 (3.1)	25.3 (7.9)	30.8 (10.9)	27.8 (4.3)
TSS, 50 mg/ℓ	64.3 (22.9)	64.8 (34.9)	90.8 (41.3)	50.5 (7.4)	57.7 (8.0)	58.3 (2.5)
TSS, 100 mg/ℓ	127.9 (39.1)	110.2 (44.0)	91.8 (38.8)	120.0 (45.6)	121.8 (46.9)	119.8 (44.6)

Note: Standard deviations are given in parentheses. TSS values are listed by treatments in order of increasing nominal values.

Gravimetrically determined total suspended sediment (TSS) values did not reflect exactly the nominal suspended sediment levels assigned to each treatment, but generally did increase in a corresponding manner with the nominal levels (Table 2). Likewise, mercury concentrations in whole (unfiltered) water increased with increasing TSS. However, soluble mercury (in filtered water) was below or near detection limits regardless of TSS. Regression equations relating these parameters are given in Table 3.

Table 3

Regression Equations Relating TSS with Nominal Suspended Sediment Levels (NomSS) and Mercury Concentrations in Whole Water (HgWhole) and Filtered Water (HgSol)

Equation	No. of Samples	Anova Statistic	Probability, P	Adjusted Coefficient of Determination, R^2 percent
TSS = 3.616 + 1.138 NomSS*	140	1,499.389	0.0001	91.51
HgWhole = -0.744 + 0.313 TSS**	140	597.249	0.0001	81.09
HgSol = 0.273 + 0.005 TSS**	140	0.038	0.8453	0

* Linear least-squares regression.
** Geometric mean regression.

Clams exposed to mercury-contaminated suspended sediment accumulated significant amounts of mercury during all of the 7-day runs compared to clams that were not exposed to suspended sediment. However, bioaccumulation of mercury from suspended sediment by killifish was significant only in Run 2. In several other runs (3, 4, and 6), fish exposed to mercury-contaminated suspended sediment appeared to have lower concentrations of mercury than fish not exposed to suspended sediment. Orthogonal contrast statistics for these comparisons, mean bioaccumulation, and standard error of the mean for fish and clams are given in Table 4. In all runs, mercury levels were significantly higher in clams than in fish.

In all runs combined, clams exhibited a significant linear increase in mercury concentration with increasing amounts of TSS, and likewise with whole water mercury (HgWhole); whereas, fish did not. Regression equations relating mercury in clams (HgClam) and in fish (HgFish) with TSS and HgWhole for all

Table 4

Comparison of Mercury Bioaccumulation in Animals Exposed to Mercury-Contaminated Suspended Sediment with That of Animals Not Exposed to Mercury-Contaminated Suspended Sediment

Organism	Run	Anova Statistic	Probability, P	Mercury Tissue Concentration Mean (Standard Error, No. of Samples) ppm: No Suspended Sediment Exposure	Mercury Tissue Concentration Mean (Standard Error, No. of Samples) ppm: Suspended Sediment Exposure
Clams	1	25.09	0.0001**	0.145 (0.0134, 12)	0.199 (0.0077, 18)
	2	39.73	0.0001**	0.153 (0.0071, 12)	0.209 (0.0059, 18)
	3	32.40	0.0001**	0.133 (0.0072, 12)	0.195 (0.0089, 18)
	4	27.97	0.0001**	0.132 (0.0071, 11)	0.208 (0.0097, 17)
	5	53.56	0.0001**	0.107 (0.0126, 12)	0.208 (0.0089, 18)
	6	212.19	0.0001**	0.083 (0.0066, 12)	0.189 (0.0058, 18)
Fish	1	1.64	0.2145NS	<0.026 (0.0028, 12)+	<0.035 (0.0057, 18)+
	2	15.43	0.0008**	<0.024 (0.0014, 12)+	<0.038 (0.0026, 18)+
	3	2.36	0.1405NS	0.053 (0.0033, 12)	0.046 (0.0027, 18)
	4	0.95	0.3419NS	<0.011 (0.0013, 9)+	<0.010 (0.0003, 18)+
	5	0.76	0.3942NS	<0.012 (0.0009, 12)+	<0.014 (0.0024, 18)+
	6	2.85	0.1067NS	<0.016 (0.0043, 12)+	<0.010 (0.0000, 18)+

Note: In the Probability column, NS indicates not significant at $P > 0.05$ and a double asterisk indicates highly significant at $P < 0.01$.

+ Means include values below detection limits that were set equal to the detection limits for inclusion in the data analysis.

runs combined are given in Table 5. Based on the regressions, clam tissue residues of mercury increase by about 1 part per billion (ppb) for each increase in TSS of 1 mg/ℓ (ppm), or by about 4 ppb for each increase in HgWhole of 1 ppb. However, changes in TSS or whole water mercury levels accounted for only 12 to 13 percent of the variation in clam tissue residues of mercury after the 7-day exposures, as evidenced by the adjusted coefficient of determination (R^2) values.

To assess the effects of temperature and salinity on mercury uptake in clams and fish, ancovas were run comparing bioaccumulation at the two temperatures (12° and 25° C) and at the three salinities (0.5, 2, and 6 ppt). TSS and HgWhole were each used as covariates in order to statistically remove any variation in bioaccumulation due to variation in these parameters. Any significant variation in bioaccumulation that remains can then be attributed to

Table 5

Geometric Mean Regression Equations Relating Mercury in Clams (HgClam) and Fish (HgFish) with TSS and with Mercury Concentrations in Whole Water (HgWhole)

Equation	No. of Samples	Anova Statistic	Probability, P	Adjusted Coefficient of Determination R^2 percent
HgClam = 0.146 + 0.00117 TSS	139	21.518	0.0001	12.94
HgClam = 0.145 + 0.00367 HgWhole	139	19.125	0.0001	11.61
HgFish = 0.009 + 0.00047 TSS	139	1.014	0.3157	0.01
HgFish = 0.009 + 0.0015 HgWhole	139	0.050	0.8238	0

the environmental factors of interest, temperature or salinity.

After statistical adjustment for the covariates, differences in bioaccumulation between the two temperatures were not significant for either fish or clams. Both organisms experienced slightly increasing mercury uptake with increasing salinity, but a significant difference was noted only for clams after adjusting for HgWhole as a covariate. In this case, clams exhibited significantly higher mercury concentrations at 6 ppt than at 0.5 ppt salinity. The mean tissue concentrations of mercury (not adjusted for covariates) in clams and fish following exposure to the various nominal TSS levels are shown in Figure 1 for the two temperatures, and in Figure 2 for the three salinities. Differences between the two organisms are far more apparent than any differences due to temperature, salinity, or TSS.

Discussion and Conclusions

Mercury uptake by killifish was clearly not influenced by temperature, salinity, or concentration of mercury-contaminated suspended sediment in this experiment. The fish simply did not bioaccumulate mercury under the conditions of exposure. It would appear that the sediment-associated mercury was not bioavailable to these estuarine fish under the experimental conditions. These results are consistent with those of Weis, Weis, and Bogden (1986), who

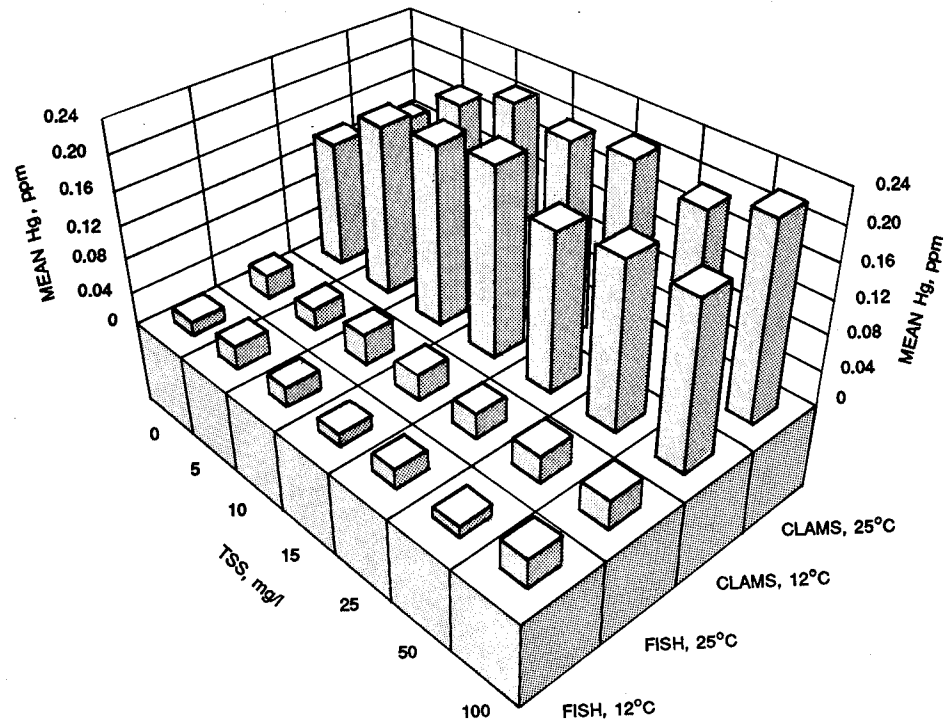

Figure 1. Mean mercury concentrations (ppm) in tissues of fish and clams exposed to the seven TSS treatments (0, 5, 10, 15, 25, 50, and 100 mg/ℓ) at two temperatures (12° and 25° C)

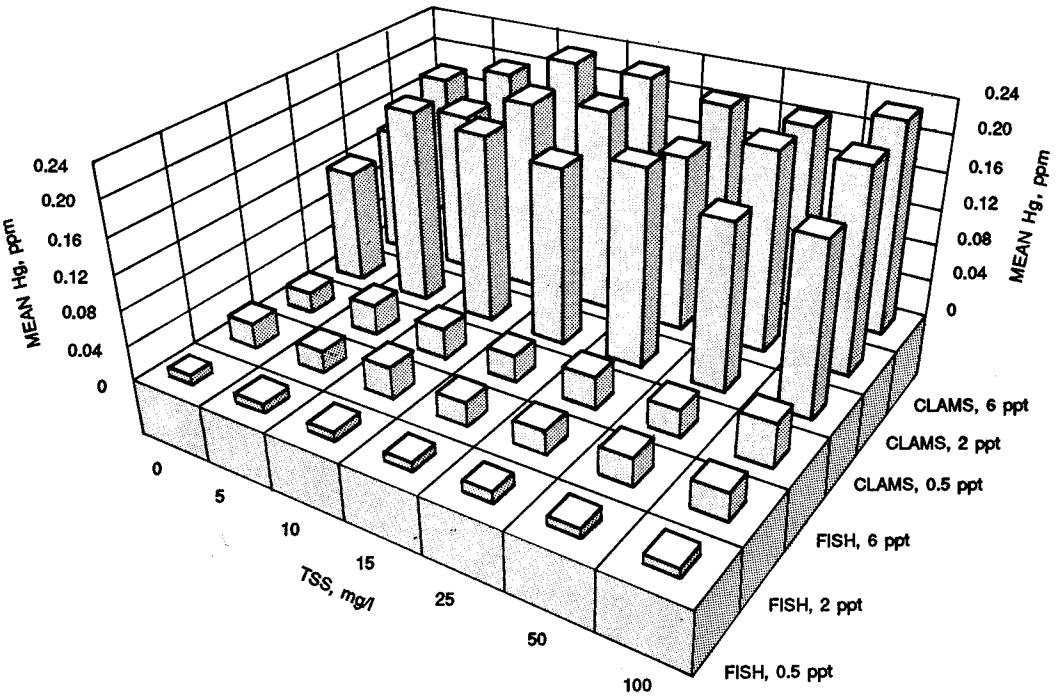

Figure 2. Mean mercury concentrations (ppm) in tissues of fish and clams exposed to the seven TSS treatments (0, 5, 10, 15, 25, 50, and 100 mg/ℓ) at three salinities (0.5, 2, and 6)

found no relationship between mercury uptake in killifish and salinity or mercury concentration in sediment to which the fish were exposed.

Clams, on the other hand, were able to accumulate mercury in this experiment, and had consistently higher mercury tissue residues than the fish. Mercury bioaccumulation in the clams appeared to be slightly enhanced by increasing salinity and increasing concentrations of mercury-contaminated suspended sediment. However, mercury tissue residues were not significantly enhanced at the higher temperature compared to the lower temperature.

Mercury content of the sediment from which the suspended particulate slurries were prepared ranged from about 80 to 100 ppm. This was two to three orders of magnitude greater than the mercury concentrations in tissues of animals exposed to those slurries. Clearly even the clams did not bioaccumulate mercury to any great extent in this experiment. The absence of detectable mercury in filtered water samples suggests that the mercury remained tightly sorbed to the suspended sediment. Binding of mercury in the organic fraction of the sediment could thus contribute to its lack of bioavailability, especially since total sediment organic carbon was quite high, in the range of 10-11 percent. The short duration of exposure (7 days) and continuous water exchange in this experiment may have also contributed to the lack of mercury uptake by organisms.

Preliminary data indicate that sulfide levels in the sediment were very high, around 20,000 ppm. However, the high sulfide levels probably had little influence on mercury bioavailability in this experiment because sulfide is rapidly oxidized in aqueous systems in the presence of dissolved oxygen and suspended sediment.* Sulfides would likely interact with mercury to form insoluble HgS only under anaerobic conditions.

In summary, temperature and salinity had little or no impact on uptake of mercury by estuarine fish and clams in the experiment described herein. Bioaccumulation of mercury by the clams appeared to be enhanced by increasing suspended sediment concentrations, but was still extremely low considering the high mercury content of the sediment. Mercury bioavailability may have been severely limited by the high sediment organic carbon content, if the mercury remained tightly bound in the organic fraction of the suspended sediment.

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* Personal communication, Dr. James Brannon, Environmental Laboratory, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

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